REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: October 19, 2001

George E. Heibel, Ph.D.
Reg. No. 42,648

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 4, line 10, has been amended as follows:

Fig. 1 is a portion of the amino acid sequence of Semenogelin I (SEQ ID NOs:1-4) and Semenogelin II (SEQ ID NOs:5-11), showing the cleavage sites for human kallikrein 2.

Paragraph beginning at page 5, line 29, has been amended as follows:

Some examples of preferred peptides include (Note that the symbol][denotes an hK2 cleavage site):

1. Lys-Arg-Arg][(SEQ ID NO:12)
2. Ser-Arg-Arg][(SEQ ID NO:13)
3. Ala-Arg-Arg][(SEQ ID NO:14)
4. His-Arg-Arg][(SEQ ID NO:15)
5. Gln-Arg-Arg][(SEQ ID NO:16)
6. Ala-Phe-Arg][(SEQ ID NO:17)
7. Ala-Gln-Arg][(SEQ ID NO:18)
8. Ala-Lys-Arg][(SEQ ID NO:19)
9. Ala-Arg-Lys][(SEQ ID NO:20)
10. Ala-His-Arg][(SEQ ID NO:21)

Paragraph beginning at page 6, line 12, has been amended as follows:

Additional preferred peptides of longer sequence length include:

11. Gln-Lys-Arg-Arg][(SEQ ID NO:22)
12. Lys-Ser-Arg-Arg][(SEQ ID NO:23)
13. Ala-Lys-Arg-Arg][(SEQ ID NO:24)
14. Lys-Lys-Arg-Arg][(SEQ ID NO:25)
15. His-Lys-Arg-Arg][(SEQ ID NO:26)
16. Lys-Ala-Phe-Arg][(SEQ ID NO:27)
17. Lys-Ala-Gln-Arg][(SEQ ID NO:28)

18. Lys-Ala-Lys-Arg][(SEQ ID NO:29)
19. Lys-Ala-Arg-Lys][(SEQ ID NO:30)
20. Lys-Ala-His-Arg][(SEQ ID NO:31)

Paragraph beginning at page 6, line 24, has been amended as follows:

Additional preferred peptides that include an X-1 amino acid are:

21. Lys-Arg-Arg][Leu (SEQ ID NO:32)
22. Ser-Arg-Arg][Leu (SEQ ID NO:33)
23. Ala-Arg-Arg][Leu (SEQ ID NO:34)
24. Ala-Arg-Arg][Ser (SEQ ID NO:35)
25. His-Arg-Arg][Ala (SEQ ID NO:36)
26. Gln-Arg-Arg][Leu (SEQ ID NO:37)
27. Ala-Phe-Arg][Leu (SEQ ID NO:38)
28. Ala-Gln-Arg][Leu (SEQ ID NO:39)
29. Ala-Lys-Arg][Leu (SEQ ID NO:40)
30. Ala-Arg-Lys][Leu (SEQ ID NO:41)
31. Ala-His-Arg][Leu (SEQ ID NO:42)

Paragraph beginning at page 7, line 7, has been amended as follows:

Preferred peptides of still longer sequence length having X_{-1} include:

32. His-Ala-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:43)
33. Gly-Gly-Lys-Ser-Arg-Arg][Leu (SEQ ID NO:44)
34. His-Glu-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:45)
35. His-Glu-Ala-Lys-Arg-Arg][Leu (SEQ ID NO:46)
36. Gly-Gly-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:47)
37. His-Glu-Gln-Lys-Arg-Arg][Ala (SEQ ID NO:48)
38. Gly-Gly-Ala-Lys-Arg-Arg][Leu (SEQ ID NO:49)
39. His-Glu-Gln-Lys-Arg-Arg][Ser (SEQ ID NO:50
40. Gly-Gly-Lys-Lys-Arg-Arg][Leu (SEQ ID NO:51)
41. Gly-Gly-His-Lys-Arg-Arg][Leu (SEQ ID NO:52)

Paragraph beginning at page 15, line 28, has been amended as follows:

Recombinant hK2 was produced and purified as described in Lövgren et al., *Biochem. Bioph. Res. Co.*, 238, 549-555 (1997). Semenogelin I and II were isolated from human semen as described previously in Malm et al., *Eur. J. Biochem.*, 238, 48-53 (1996). The tripeptide aminomethylcoumarin (AMC) substrates [Bos] Boc-Phe-Ser-Arg-AMC (SEQ ID NO:53), Boc-Gln-Gly-Arg-AMC (SEQ ID NO:54), H-Pro-Phe-Arg-AMC (SEQ ID NO:55), boc-Val-Pro-Arg-AMC (SEQ ID NO:56), H-D-Val-Leu-Lys-AMC (SEQ ID NO:57), Tos-Gly-Pro-Arg-AMC- (SEQ ID NO:58), Tos-Gly-Pro-Lys-AMC (SEQ ID NO:59), Z-Leu-Leu-Arg-AMC (SEQ ID NO:60), Z-Val-Val-Arg-AMC (SEQ ID NO:61), Z-Ala-Arg-Arg-AMC (SEQ ID NO:62), and H-Arg-Gln-Arg-Arg-AMC (SEQ ID NO:63) were from Bachem (Bubendorf, Switzerland). The heptapeptide substrates Mu-Ala-Pro-Val-Leu-Ile-Leu-Ser-Arg-AMC (SEQ ID NO:64) and Mu-Val-Pro-Leu-Ile-Gln-Ser-Arg-AMC (SEQ ID NO:65) corresponding to the pro peptides of PSA hK2 were from Enzyme Systems Product (Livermore, CA, USA). ACT was purified from human blood plasma as described in Christenssson et al., *Eur. J. Biochem.*, 194, 755-63 (1990). PCI was provided by Prof. Johan Stenflo (Malmö University Hospital, Malmö, Sweden), and SLPI, and PSTI by Prof. Kjell Ohlsson (Malmö University Hospital, Malmö, Sweden). Benzamidine hydrochloride was from Amresco® (Solon, OH, USA), leupeptin and antipain were from ICN Biomedicals (Costa Mesa, CA, USA), Aprotinin was from Sigma (St. Louis, MO, USA), and PPACK from Calbiochem (La Jolla, CA, USA).

Paragraph beginning at page 18, line 10, has been amended as follows:

Substrates ending in either arginine or lysine were tested. The kinetic constants for hydrolysis of the substrates by hK2 are shown in Table 1. The best substrate was the kallikrein substrate Pro-Phe-Arg-AMC (SEQ ID NO:55) having the highest kcat and k_{cat}/K_m values. The cathepsin B substrate Ala-Arg-Arg-AMC (SEQ ID NO:62) was also cleaved quite effectively having a relatively high k_{cat} value and a low K_m resulting in a four times lower k_{cat}/K_m value than that obtained for the kallidrein substrate Pro-Phe-Arg-AMC (SEQ ID NO:55). However, no hydrolysis of Arg-Gln-Arg-Arg-AMC (SEQ ID NO:63) was detected. HK2 cleaved additionally

Val-Pro-Arg-AMC (SEQ ID NO:56), and Leu-Leu-Arg-AMC (SEQ ID NO:60), but with lower efficiency. As with the semenogelins hK2 also here cleaves substrates with Arg at position P1 and preferentially a large residue or another Arg at position P2. None of the substrates with lysine in the C-terminal position were cleaved.

Table 1 beginning at page 19, line 1, has been amended as follows:

Table 1. Substrate Hydrolysis by hK2

Substrates	Km (M)	Kcat (min^{-1})	Kcat/km ($\mu M^{-1}min^{-1}$)	Activity (%)
Pro Phe Arg-AMC (SEQ ID NO:55)	40	55	1.375	100
Val Pro Arg-AMC (SEQ ID NO:56)	48	1.6	0.034	6
Gly Pro Arg-AMC (SEQ ID NO:58)		NR		
Gly Pro Lys-AMC (SEQ ID NO:59)		NR		
Leu Leu Arg-AMC (SEQ ID NO:60)	71	2.4	0.034	7
Val Val Arg-AMC (SEQ ID NO:61)		NR		
Val Leu Lys-AMC (SEQ ID NO:57)		NR		
Phe Ser Arg-AMC (SEQ ID NO:53)		NR		
Gln Gly Arg-AMC (SEQ ID NO:54)		NR		
Ala Arg Arg-AMC (SEQ ID NO:62)	20	7.2	0.360	33
Arg Gln Arg Arg-AMC (SEQ ID NO:63)		NR		

Paragraph beginning at page 19, line 3, has been amended as follows:

The activity listed in Table 1 is the hydrolytic activity of hK2 with 100 μM substrate in relation to the hydrolytic activity of hK2 with 100 μM of the tissue kallikrein substrate H-Pro-Phe-[arg] Arg-AMC (SEQ ID NO:55). The entry "N.R." means that no reaction was detected.

Paragraph beginning at page 19, line 8, has been amended as follows:

Activity of hK2 (1.6 pmol) was monitored using the substrate H-Pro-[Lphe] Phe-[arg] Arg-AMC (SEQ ID NO:55) (90 μM). Inhibitors, at commonly used concentrations, and hK2 (8.3 nM) were mixed and proteolysis of 90 μM H-Pro-Phe-Arg-AMC (SEQ ID NO:55) was followed up to 20 minutes, starting directly or 10 minutes after mixing the enzyme with various inhibitors. Inhibition was evaluated by comparison with enzyme-free controls.

Paragraph beginning at page 22, line 27, has been amended as follows:

The progress of the reaction of hK2 (8nM final concentration) with the substrate Pro-Phe-Arg-AMC (SEQ ID NO:55) was monitored at two different substrate concentrations without or with different concentrations of PCI (80, 40 or 16 nM final concentration). The fluorescence measurements were started directly after mixing the enzyme with the inhibitor. The inhibitor of hK2 by PCI could be described by the slow-binding inhibition mechanism presented in Scheme 2, which has been used in analyzing the interaction of PCI with various serine proteases (Hermans et al., *Biochem. J.*, 295, 239-245 (1993), and Hermans et al., *Biochemistry*, 33, 5440-44 (1994)). This mechanism assumes that a reversible complex is formed between the proteinase and serine proteinase inhibitor (serpin). The issues justifying the use of the slow binding inhibition mechanism despite the commonly held view that the seprin-proteinase complex is irreversible has been discussed in more detail by Hermans et al. (1993).

Table 4 beginning at page 25, line 1, has been amended as follows:

Table 4. Hydrolysis of hK2 Substrates

Peptide Sequence									hK2 Hydrolysis Rate (FU/hr/mg)	Serum Hydrolysis Rate FU/hr
P7	P6	P5	P4	P3	P2	P1	P'1			
G	H	E	Q	K	R	R	L	(SEQ ID NO:66)	5966.31	0.17
G	G	G	K	A	R	R	L	(SEQ ID NO:67)	4784.22	0.03
G	G	G	K	A	H	R	L	(SEQ ID NO:68)	4100.94	0.09
G	P	A	H	Q	R	R	L	(SEQ ID NO:69)	4017.81	0.10
G	S	K	G	H	F	R	L	(SEQ ID NO:70)	3029.27	0.04
G	S	K	G	H	R	R	L	(SEQ ID NO:71)	2649.96	UD
G	K	D	V	S	R	R	L	(SEQ ID NO:72)	2316.12	0.08
G	S	Q	N	Q	R	R	L	(SEQ ID NO:73)	2100.48	0.05
G	S	Y	P	S	R	R	L	(SEQ ID NO:74)	2060.21	0.09
G	S	Y	P	S	S	R	L	(SEQ ID NO:75)	1456.18	0.06
G	H	E	Q	K	G	R	L	(SEQ ID NO:76)	650.80	0.04
G	S	N	T	E	R	R	L	(SEQ ID NO:77)	592.34	UD
G	S	Y	E	E	R	R	L	(SEQ ID NO:78)	324.75	0.04
G	K	D	V	S	G	R	L	(SEQ ID NO:79)	242.91	0.05
G	S	N	T	E	K	R	L	(SEQ ID NO:80)	255.90	0.13
G	S	K	G	H	F	H	L	(SEQ ID NO:81)	171.47	0.10
G	S	Q	N	Q	V	R	L	(SEQ ID NO:82)	193.55	0.03
G	P	L	I	L	S	R	L	(SEQ ID NO:83)	118.21	0.07
G	S	Y	E	E	R	H	L	(SEQ ID NO:84)	42.87	0.09
G	K	D	V	S	G	H	L	(SEQ ID NO:85)	67.55	0.05
G	G	G	K	A	H	H	L	(SEQ ID NO:86)	70.15	0.05
G	S	N	T	E	K	H	L	(SEQ ID NO:87)	80.54	0.03
G	P	A	H	Q	D	R	L	(SEQ ID NO:88)	75.34	0.06
G	H	E	Q	K	G	H	L	(SEQ ID NO:89)	1.30	UD
G	P	A	H	Q	D	H	L	(SEQ ID NO:90)	48.06	0.00
G	S	Y	P	S	S	H	L	(SEQ ID NO:91)	24.68	UD
G	S	Q	N	Q	V	H	L	(SEQ ID NO:92)	32.48	0.03

Table 5 beginning at page 26, line 1, has been amended as follows:

Table 5. Additional hK2 Substrates

Substrate Sequence									hK2 Hydrolysis Rate(FU/hr/mg)	Serum Hydrolysis Rate FU/hr
P7	P6	P5	P4	P3	P2	P1	P'1			
G	H	A	Q	K	R	R	L	(SEQ ID NO:93)	3665.1	0.08
	G	G	K	S	R	R	L	(SEQ ID NO:94)	3439.7	0.03
G	H	E	Q	K	R	R	L	(SEQ ID NO:66)	3366.5	UD
G	H	E	A	K	R	R	L	(SEQ ID NO:95)	3324.1	UD
	G	G	Q	K	R	R	L	(SEQ ID NO:96)	3267.4	0.02
G	H	E	Q	K	R	R	A	(SEQ ID NO:97)	3051.5	0.06
	G	G	A	K	R	R	L	(SEQ ID NO:98)	2773.0	0.02
G	H	E	Q	K	R	R	S	(SEQ ID NO:99)	2638.5	UD
	G	G	K	K	R	R	L	(SEQ ID NO:100)	2583.0	UD
	G	G	H	K	R	R	L	(SEQ ID NO:101)	2428.4	UD
	G	G	K	A	F	R	L	(SEQ ID NO:102)	2374.2	0.07
G	A	E	Q	K	R	R	L	(SEQ ID NO:103)	2325.8	0.10
	G	G	K	A	Q	R	L	(SEQ ID NO:104)	2233.7	0.04
	G	G	K	A	R	R	L	(SEQ ID NO:105)	2171.2	UD
	G	G	K	Q	R	R	L	(SEQ ID NO:106)	2171.2	0.02
	G	G	K	H	R	R	L	(SEQ ID NO:107)	2079.2	UD
G	H	E	Q	A	R	R	L	(SEQ ID NO:108)	1956.4	0.14
	G	G	K	A	K	R	L	(SEQ ID NO:109)	1788.9	0.14
G	H	E	Q	K	R	R	dL	(SEQ ID NO:110)	1690.9	0.15
	G	G	K	A	R	R	S	(SEQ ID NO:111)	1609.5	UD
	G	G	K	A	R	K	L	(SEQ ID NO:112)	1602.4	UD
G	H	E	Q	K	R	R	E	(SEQ ID NO:113)	1473.8	UD
	G	G	K	A	H	R	L	(SEQ ID NO:114)	1287.4	0.10
	G	G	K	A	N	R	L	(SEQ ID NO:115)	1113.9	0.01
	G	G	K	A	R	Q	L	(SEQ ID NO:116)	1021.9	0.13
	G	G	K	A	R	H	L	(SEQ ID NO:117)	939.3	UD
	G	G	K	A	R	N	L	(SEQ ID NO:118)	828.4	0.25
	G	G	K	A	dR	R	L	(SEQ ID NO:119)	494.4	0.06
	G	G	K	A	K	K	L	(SEQ ID NO:120)	77.9	UD
	G	G	K	A	H	K	L	(SEQ ID NO:121)	73.2	UD
	G	G	K	A	R	dR	L	(SEQ ID NO:122)	49.6	UD
	G	G	K	A	dR	dR	L	(SEQ ID NO:123)	16.5	UD

In the claims:

Claims 8, 28, and 29 have been amended as follows:

8. (Amended) The peptide of claim 6, wherein the amino acid sequence is selected from the group consisting of Ala-Gln-Lys-Arg-Arg (SEQ ID NO:124), Gly-Lys-Ser-Arg-Arg (SEQ ID NO:125), Glu-Gln-Lys-Arg-Arg (SEQ ID NO:126), Glu-Ala-Lys-Arg-Arg (SEQ ID NO:127), Gly-Gln-Lys-Arg-Arg (SEQ ID NO:128), Gly-Ala-Lys-Arg-Arg (SEQ ID NO:129), Gly-Lys-Lys-Arg-Arg (SEQ ID NO:130), Gly-His-Lys-Arg-Arg (SEQ ID NO:131), Gly-Lys-Ala-Phe-Arg (SEQ ID NO:132), Glu-Lys-Ala-Gln-Arg (SEQ ID NO:133), and Glu-Lys-Ala-Arg-Arg (SEQ ID NO:134).

28. (Amended) The composition of claim 17, wherein the peptide is Gly-Gly-Lys-Ala-Arg-Arg-Leu (SEQ ID NO:135).

29. (Amended) The composition of claim 17, wherein the therapeutic drug is a compound belonging to the group of thapsigargins which have been derivatized with a moiety containing a primary amine group, the peptide is Gly-Gly-Lys-Ala-Arg-Arg-Leu (SEQ ID NO:135), and the linker is selected from the group consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkenyl-, amido- or amino-substituted CO-$(CH=CH)_{n1}$-$(CH_2)_{n2}$-Ar-NH_2, CO-$(CH_2)_{n2}$-$(CH=CH)_{n1}$-Ar-NH_2, CO-$(CH_2)_{n2}$-$(CH=CH)_{n1}$-CO-NH-Ar-NH_2, CO-$(CH=CH)_{n1}$-$(CH_2)_{n2}$-CO-NH-Ar-NH_2, CO-$(CH_2)_{n3}$-NH_2, and CO-$(CH_2)_{n3}$-NH-CO-$CH(R_4)$-NH_2, wherein n1 and n2 are from 0 to 5, n3 is from 0 to 15, Ar is any substituted or unsubstituted aryl group, attachment of NH_2 to Ar is in a ortho, meta or para position with respect to the remainder of the linker, and R_4 is any naturally occurring amino acid side chain.